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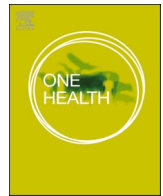
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# Molecular prevalence of *Coxiella burnetii* in bulk-tank milk from bovine dairy herds: Systematic review and meta-analysis

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## ABSTRACT

*Coxiella burnetii* is an obligate intracellular zoonotic bacterium that causes Q fever. Ruminants, including cattle, are broadly known to be reservoirs for this bacterium. Since 2006, many research groups have evaluated the herd-level prevalence of *C. burnetii* in cattle by molecular techniques on composite milk samples. This study explored the global *C. burnetii* herd-level prevalence from studies done on bovine bulk-tank milk (BTM) samples using PCR-based analysis. Also, moderators were investigated to identify sources of heterogeneity. Databases (CAB Abstracts, Medline via Ovid, PubMed, Web of Science and Google Scholar) were searched for index articles on *C. burnetii* prevalence in BTM samples by PCR published between January-1973 and November-2018. Numerous studies (1054) were initially identified, from which seventeen original publications were included in the meta-analysis based on the pre-defined selection criteria. These studies comprised 4031 BTM samples from twelve countries. A random-effects model was used because of considerable heterogeneity ( $I^2 = 98\%$ ) to estimate the herd-level prevalence of *C. burnetii* as 37.0% (CI<sub>95%</sub> 25.2–49.5%). The average herd size appeared to account for a high level of the heterogeneity. No other moderators (geographic location, gross national income or notification criteria for Q fever) seemed to be determinant. This systematic evaluation demonstrated a high molecular prevalence of *C. burnetii* in BTM samples both in European and non-European countries, evidencing a widespread herd-level circulation of this agent in bovine dairy farms around the world. Meta-regression showed herd size as the most relevant moderator with the odds of a BTM sample testing positive doubling with every unit increase.

## 1. Introduction

*Coxiella burnetii* the intracellular Gram-negative bacterium responsible for the zoonotic disease Q fever [1] has many reservoirs, including ruminants, that represent the primary source of environmental contamination and of infection in people [2]. This agent causes fertility disorders and metritis in cattle and is implicated in bovine abortion [3–5]. It often leads to abortion in small ruminants when a pregnant dam is infected, as *C. burnetii* exhibits a specific tropism for the trophoblast cells in placental cotyledons [6].

*Coxiella burnetii* has a complex epidemiological pattern and characteristics that make its control challenging. It is widely disseminated in nature and infects a large number of species, including mammals, birds, reptiles and fish [7]. There are two maintenance cycles in nature, one

involving domestic species, and another including wild animal species and their ectoparasites. Ticks may be involved in the transmission of *C. burnetii* between wildlife and domestic species [8]. Additionally, the agent is extremely resistant remaining viable in the environment over extended periods [8]. *Coxiella burnetii* can also undergo air-borne transmission by contaminated dust particles, which can be facilitated by hot and dry weather conditions [9,10].

A large human outbreak of Q fever reported in the Netherlands (2007–2010), comprising more than 4000 cases, emphasised the need for robust surveillance campaigns and highlighted its importance as a threat to public health [9,11]. Transmission to people is principally by the inhalation of aerosolised contaminated animal placenta and birth fluids during abortions or the birth of normal offspring [12]. Practices such as the assistance of calving, handling of birth products, and manure

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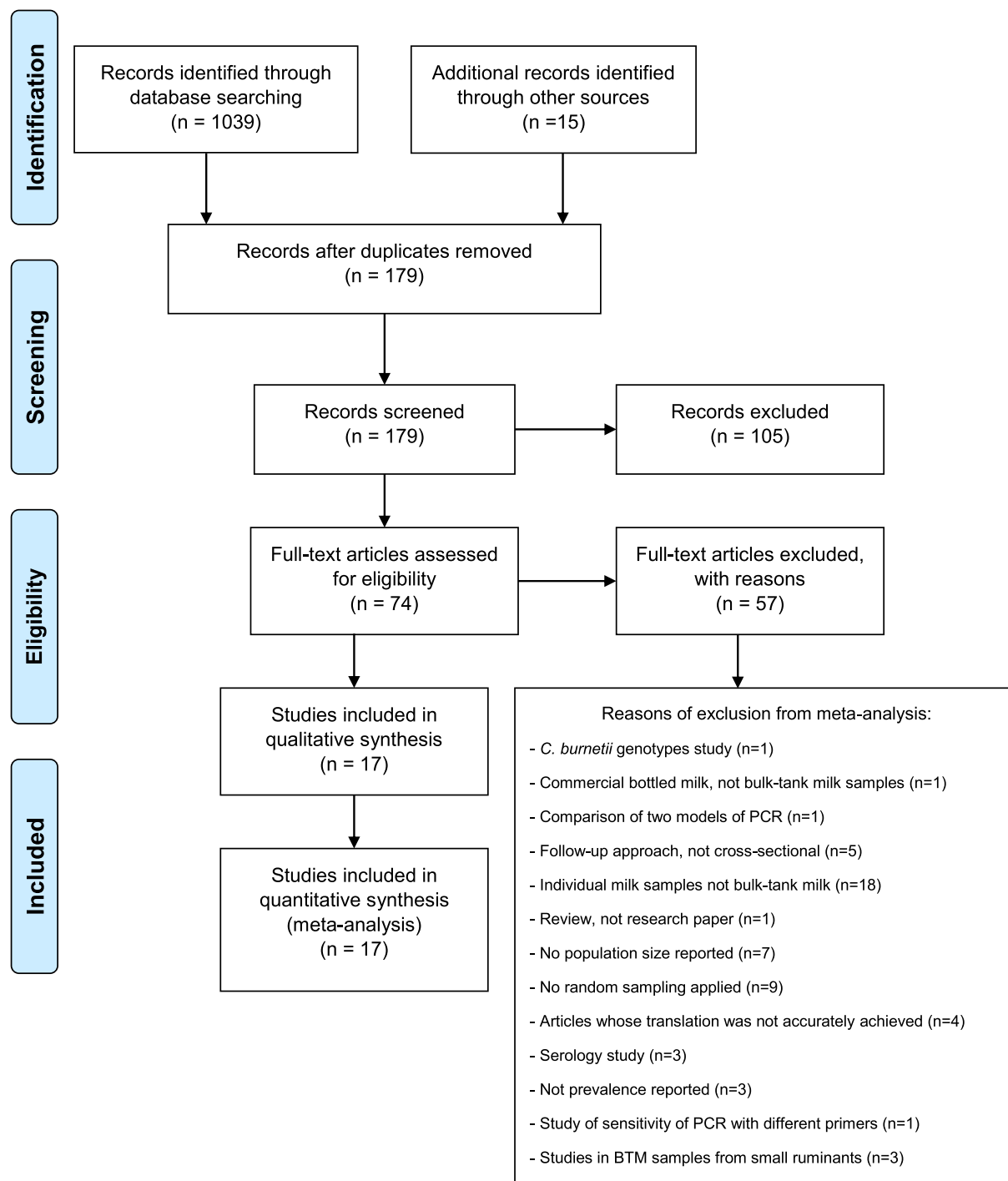
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spreading may present a high risk for *C. burnetii* transmission to humans [13–15]. There is no consensus about the importance or effectiveness of the digestive route of infection by the consumption of raw milk and dairy product [6,16–18]. Nevertheless, respiratory exposure to aerosols produced during milking of animals should not be underestimated [19].

The level of bacterial load by the different routes differs among ruminants [6]. While parturition products are the primary source of shedding in small ruminants, milk seems to play a central role as a shedding route of *C. burnetii* in dairy cattle [20,21]. Even asymptomatic animals [20] or seronegative cattle [22] have been identified as

*C. burnetii* milk shedders. *Coxiella burnetii* can be excreted in milk for up to 13 months [9,23], although this may be intermittent [6]. Two patterns of shedding have been identified in dairy cows which can be persistent heavy shedders or sporadic shedders [20].

Based on these heterogeneous shedding patterns, composite samples such as bulk-tank milk (BTM) constitute useful and easily accessible specimens for large scale epidemiological investigation. A positive result provides robust evidence for the identification of infected herds. Bulk-tank milk testing is the preferred diagnostic approach for disease notification in many countries [24] and has epidemiological value for the



**Fig. 1.** PRISMA flow diagram describing the study design process for the systematic review and meta-analysis of the molecular prevalence of *Coxiella burnetii* in bulk-tank milk from bovine dairy herds.

monitoring of infection status over time in follow-up evaluations [25].

Recent large human Q fever outbreaks in the Netherlands, Spain, France and Germany have increasingly focussed attention on coxiellosis in many European countries where strategies including mandatory notification of the disease have been implemented. We systematically review studies of the herd prevalence of *C. burnetii* in dairy cattle using PCR on BTM samples, conduct a meta-analysis to determine the overall European and global prevalences and assess geographic region, average herd size, local legislation for coxiellosis and per capita income in each country where studies were conducted as potential moderators.

## 2. Material and methods

### 2.1. Literature search and study selection

The systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26] (Fig. 1). The search strategy identified publications reporting the prevalence of *C. burnetii* on BTM samples analysed by molecular studies. The following electronic databases were used to identify studies published from January 1973 up to November 2018 (week 43 of 2018): CAB Abstracts, Medline, PubMed, Web of Science, Scopus, Science Direct and Google Scholar. The literature search comprised the terms: “*Coxiella burnetii*” or “Q fever” or “coxiellosis” and “PCR” or “qPCR” or “real-time PCR” or “molecular diagnosis” and “BTM” or “milk”, with no language restriction. No constraint in study designs was applied at this phase. Additional publications were identified by cross checking references included in the articles. Duplicates were identified by reference management software (Mendeley) and manually removed.

### 2.2. Eligibility - inclusion criteria

Publications on studies fulfilling all the following criteria were eligible for inclusion: (i) molecular investigation of *C. burnetii* by PCR, (ii) random sampling, (iii) composite single test-day samples obtained from the bulk storage tank located on a dairy cattle farm, (iv) primary studies, but not reviews, (v) cross-sectional studies reporting prevalence. Authors of articles not stating the total number of dairy cattle herds from which the sample was drawn were contacted to provide this missing data. Publications were examined by two independent reviewers (AR and MF) to ensure they matched the inclusion criteria. Discrepancies between the two reviewers on eligibility were discussed with the rest of authors until reaching agreement.

### 2.3. Data extraction and meta-analysis

Studies were screened by title, and abstract and irrelevant publications were excluded. The remaining studies were full-text checked against the inclusion criteria described above. Articles that did not fulfil all these criteria were excluded. The number of publications excluded are shown in Fig. 1. Data were systematically extracted from all the studies that satisfied the inclusion criteria, including: the first author identity, year of publication, study title, journal title, country, study methodology (duration of sampling, herd size, sample size, the number of positives herds and/or prevalence, randomisation), molecular technique and target gene used. When available, information about the factors associated with the *C. burnetii* infection was also reported.

The *C. burnetii* herd prevalence determined in BTM samples (dependant variable) was considered as the effect size for the studies included in the meta-analysis. This meta-analysis of proportions was performed as outlined by Wang [27]. The heterogeneity among studies was first investigated by Cochran's  $Q$  ( $X^2$ ) that tests the null hypothesis of homogeneity, and then quantified by the Higgins'  $I^2$  statistic [28]. The heterogeneity was measured to select the model for the overall weighted *C. burnetii* herd prevalence estimation. As the level of heterogeneity was

high, a random-effects model was first used to address both within-study variance (the sampling error) and the between-studies variance ( $\tau^2$ ). Possible sources of heterogeneity were investigated through the analysis of moderators. The evaluated moderators included: i) geographic region: Europe vs non-Europe; ii) average herd size; iii) local legislation for Q fever: mandatory notification vs non-mandatory notification [29–37], and iv) gross national income (GNI) per capita classification from the year the study was conducted, based on the Atlas method [38]. A subgroup analysis was performed for the categorical moderators. Categorical moderators were analysed using a mixed-effects model. The statistical significance of the moderators was evaluated by an omnibus test ( $QM$ ) within the mixed-effects model [39]. The proportion of heterogeneity accounted for by each moderator was explored by the  $R^2$  index. Meta-regression was also utilised to explore heterogeneity among the studies. All the moderators and their interactions were entered in the initial model and non-significant terms were then dropped stepwise (from lowest  $R^2$  to highest  $R^2$ ) [40]. The odds ratio (OR) for  $\log_e$  average herd size was additionally investigated. Association among moderators was assessed by the Pearson correlation coefficient ( $r$ ). Results from the meta-analysis with the corresponding 95% confidence intervals were summarized using forest plots. Egger's test was used to test for the possibility of a publication bias for studies with low or high effect sizes [41]. All the assessments were conducted using open RStudio software (Boston, MA) with metafor package, mvmeta package and metaprop commands [39,42].

## 3. Results

### 3.1. Description of the studies

After removal of duplicates, a total of 179 studies were identified initially (Fig. 1). Seventeen studies from twelve different countries (Belgium, Colombia, Hungary, Iran [2 studies], Italy [3 studies], Latvia, Netherlands [2 studies], Portugal, Spain, South Korea, UK and USA [2 studies]) were eligible for the meta-analysis based on the inclusion criteria. Six of those studies were conducted in non-European countries and 11 in European countries; 10 were conducted in countries where Q fever is a notifiable disease, while 7 were from countries where it is not. The study conducted in the Basque Country was included in the subgroup with mandatory notification, although this is the only Spanish province where the notification for Q fever is compulsory. Finally, 3 studies were conducted in upper-middle-income countries and 14 studies were in high income countries. The seventeen selected articles are summarized in Table 1 and included test results for a total of 4031 BTM samples collected over 9 years (2006 to 2015). Studies employed either conventional PCR ( $n = 5$ ), quantitative PCR ( $n = 9$ ) or nested PCR ( $n = 3$ ). The transposon-like repetitive region of the bacterial genome (*IS1111*) was the gene most frequently used as the target in these PCRs ( $n = 14$ ), followed by *com1* ( $n = 2$ ), *icd* ( $n = 1$ ) and 16S rRNA genes ( $n = 1$ ) (Table 1).

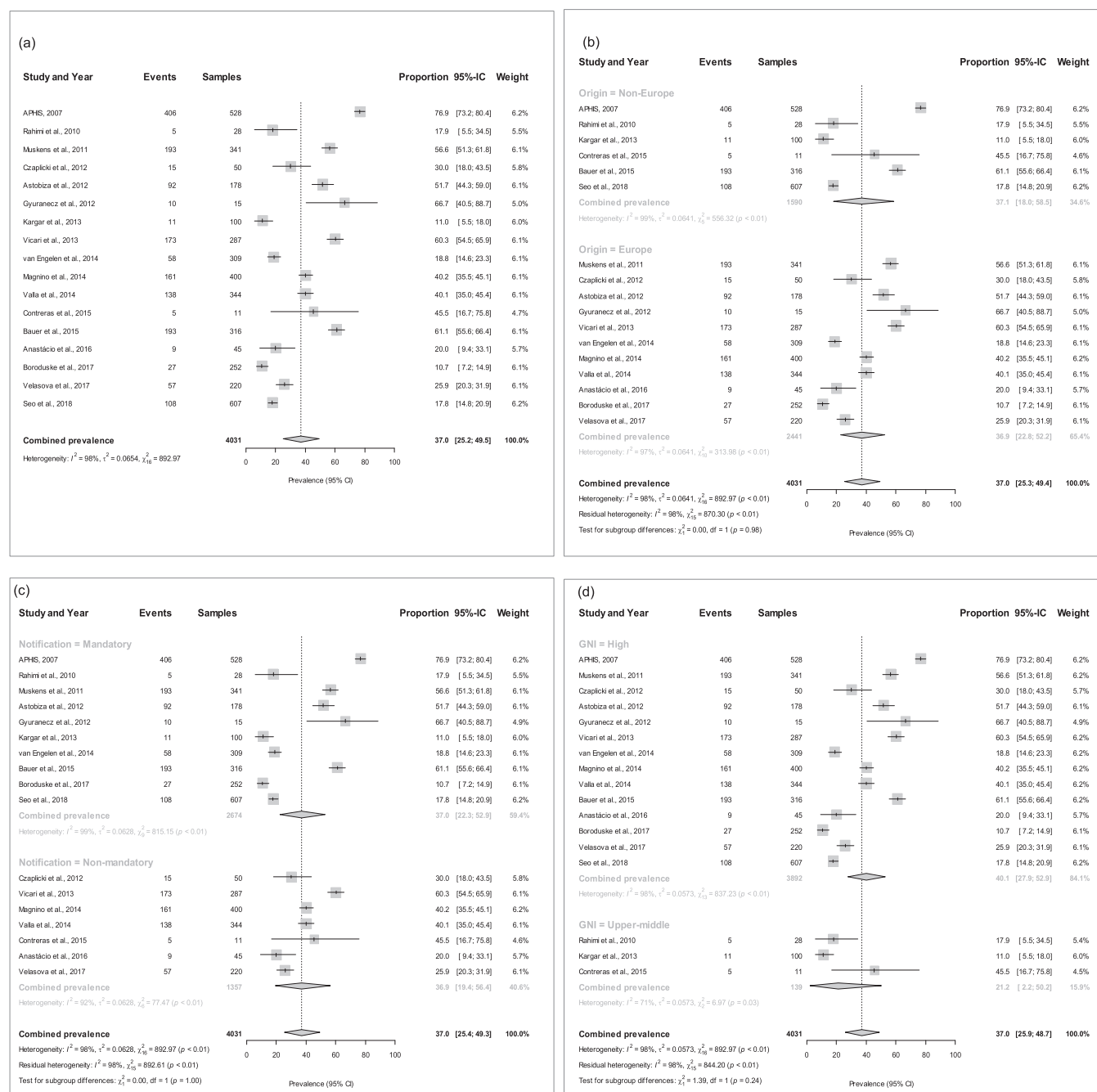
### 3.2. The estimated overall meta-prevalence of *Coxiella burnetii* in BTM samples

The median size of the eligible studies was 252 BTM samples. Of the total 4031 BTM samples, 1661 were diagnosed positive by molecular techniques. The percentages of positive BTM samples among the studies ranged from 10.7 to 76.9%. The overall weighted prevalence of *C. burnetii* in the random-effects meta-analysis was estimated at 37.0% ( $CI_{95\%} 25.2$ –49.5%). The  $I^2$  value of 98.0% ( $CI_{95\%} 95.9$ –99.0) suggested high heterogeneity, with a  $\tau^2$  of 0.0654 ( $CI_{95\%} 0.3296$ –1.4997), and an  $X^2$  statistic of 892.97 ( $P < 0.0001$ ). The overall meta-analysis is shown in a forest plot (Fig. 2a). No obvious evidence of publication bias was detected in the meta-analysis on the basis of Egger's test ( $P = 0.599$ ).

**Table 1**  
Characteristics and main results of the eligible studies ordered by molecular prevalence of *Coxiella burnetii* in bulk-tank milk samples.

Author	Year	Country	Study area	Average herd size	Period of study	Risk factor analysis	Gross national income per capita [38]	Is Q fever a mandatory notifiable disease?	Molecular approach	Target gene	N herds in study area	Percentage of herds sampled	BTM <sup>(i)</sup> samples tested	Positive BTM samples	Prevalence	95% CI
Boroduske et al. [43]	2017	Latvia	Nationwide	8.6	2015	Yes	High-income	Yes	qPCR	<i>IS1111</i>	5040	5	252	27	10.7	7.2 14.9
Kargar et al. [23]	2013	Iran	Johrom	3.7	–	Yes	Upper-middle-income	Yes	nPCR	<i>com1</i>	3000	3.3	100	11	11	5.5 18.0
Seo et al. [44]	2018	South Korea	Gyeongsang	74	2015	No	High-income	Yes	nPCR	16S rRNA	869	69.9	607	108	17.8	14.8 20.9
Rahimi et al. [45]	2010	Iran	Chaharmahal and Bakhtiari	48	2008	No	Upper-middle-income	Yes	nPCR	<i>com1</i>	95	29.5	28	5	17.9	5.5 34.5
van Engelen et al. [46]	2014	Netherlands	Nationwide	71.7	2009–2011	Yes	High-income	Yes	qPCR	<i>IS1111</i>	20,746	1.5	309	58	18.8	14.6 23.3
Anastácio et al. [47]	2016	Portugal	Nationwide	21.7	2009–2013	Yes	High-income	No	PCR	<i>IS1111</i>	1712	2.6	45	9	20	10.9 33.8
Velasova et al. [48]	2017	UK	Nationwide	133	2014–2015	No	High-income	No	qPCR	<i>icd/IS1111</i>	10,491	2.1	220	57	25.9	20.3 31.9
Czaplicki et al. [49]	2012	Belgium	Wallonia	28.5	2006	Yes	High-income	No	qPCR	<i>IS1111</i>	5086	1	50	15	30	8.7 51.3
Magnino et al. [50]	2009	Italy	Cremona, Montova and Pavia	180	2007–2008	No	High-income	No	PCR	<i>IS1111</i>	3550	11.2	400	161	40.2	35.5 45.1
Valla et al. [51]	2014	Italy	Nationwide	42.5	2011–2013	No	High-income	No	PCR	<i>IS1111</i>	30,000	1.1	344	138	40.1	35.0 45.4
Contreras et al. [37]	2015	Colombia	Monteria	150–600	2012	No	Upper-middle-income	No	PCR	<i>IS1111</i>	3341	0.3	11	5	45.5	16.7 75.8
Astobiza et al. [52]	2012	Spain	Bizkaia	46.1	2009–2010	No	High-income	No / Yes <sup>(ii)</sup>	qPCR	<i>IS1111</i>	178	100	178	92	51.7	44.4 59
Muskens et al. [25]	2011	Netherlands	Nationwide	65.7	2007	No	High-income	Yes	qPCR	<i>IS1111</i>	21,313	1.6	341	193	56.6	50.7 61.9
Vicari et al. [34]	2013	Italy	Lombardy	182	2011	No	High-income	No	PCR	<i>IS1111</i>	5750	5	287	173	60.3	54.5 65.9
Bauer et al. [53]	2015	USA	Indiana	145.3	2011	No	High-income	Yes	qPCR	<i>IS1111</i>	1225	25.8	316	193	61.1	55.6 66.4
Gyuranecz et al. [54]	2012	Hungary	Nationwide	14.5	2010–2011	No	High-income	Yes	qPCR	<i>IS1111</i>	17,172	0.1	15	10	66.7	40.5 88.7
APHIS [55]	2007	USA	18 states <sup>(iii)</sup>	162.6	2007	No	High-income	Yes	qPCR	<i>IS1111</i>	54,100	1	528	406	76.9	73.2 80.4

(i): BTM: bulk-tank milk samples, one per herd; PCR: conventional PCR; qPCR: real-time PCR; nPCR: nested PCR. (ii) mandatory notification in Basque Country. (iii) California, Idaho, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New Mexico, New York, Ohio, Pennsylvania, Texas, Vermont, Virginia, Washington, Wisconsin.



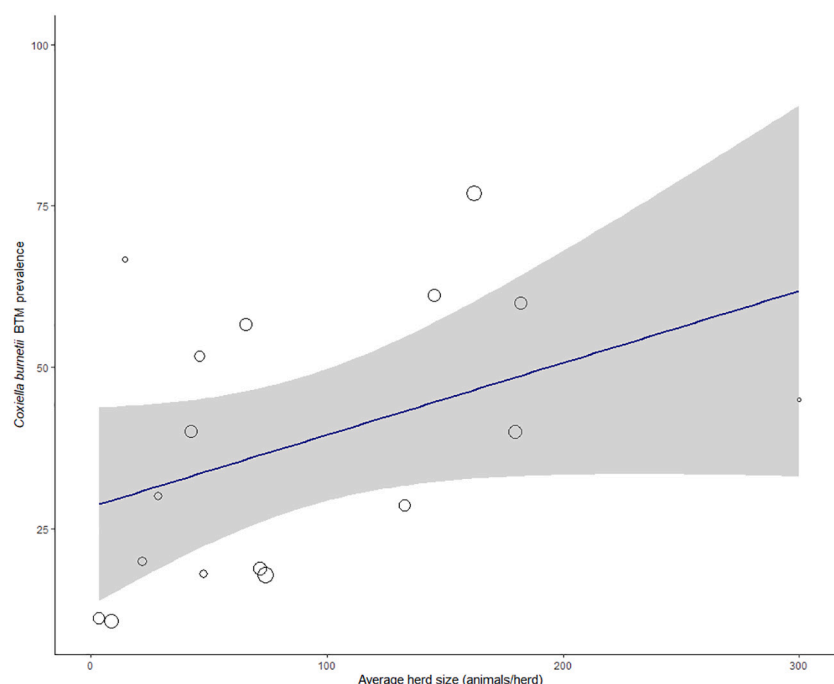
**Fig. 2.** Forest plot for the meta-analysis of herd-level *Coxiella burnetii* prevalence based on bulk-tank milk samples from the seventeen studies that matched the inclusion criteria in the systematic review. (a) All studies. (b) European and non-European country subgroups. (c) Grouped by mandatory and non-mandatory notification. (d) Grouped by the per capita Gross National Income (GNI) level.

### 3.3. The meta-prevalence of *Coxiella burnetii* and moderator analyses

The weighted average prevalence was similar within each of the two geographic subgroups (36.9% in European countries and 37.1% in non-European countries;  $I^2 = 98\%$ ;  $X^2 = 870.29$ ,  $P < 0.01$ ;  $QM$  ( $df = 1$ ) = 0.002,  $P = 0.98$ ), albeit with differing 95% confidence intervals of 22.8%–52.2% in the former and 18.0%–58.5% in the latter group of countries (Fig. 2b). Similarly, countries with mandatory and non-mandatory notification of Q fever had a prevalence around 37.0% (CI<sub>95%</sub> 22.3–52.9% and CI<sub>95%</sub> 19.4–56.4%, respectively;  $I^2 = 98\%$ ;  $X^2 = 892.61$ ,  $P < 0.01$ ;  $QM$  ( $df = 1$ ) = 0.010,  $P = 1.00$ ) (Fig. 2c). In the subgroup analysis based on the GNI per capita (Fig. 2d), the prevalence

was 40.1% (CI<sub>95%</sub> 27.9–52.9%) in high-income countries and 21.2% (CI<sub>95%</sub> 2.2–50.2%) in upper-middle-income countries ( $I^2 = 98\%$ ;  $R^2 = 3.10\%$ ;  $X^2 = 844.20$ ,  $P < 0.01$ ;  $QM$  ( $df = 1$ ) = 1.39,  $P = 0.24$ ). None of the three factors above appeared to contribute meaningfully to the observed level of heterogeneity based on the subgroup analysis. The meta-regression revealed that average herd size accounted for a significant proportion of the heterogeneity ( $I^2 = 97\%$ ;  $R^2 = 33.01\%$ ;  $X^2 = 552.23$ ,  $P < 0.01$ ;  $QM = 4.55$ ,  $P = 0.03$ ). As a significant moderator, high-size herds presented a higher herd-level *C. burnetii* BTM prevalence (Fig. 3). The odds ratio for the log<sub>e</sub> of herd size was 2.00 (CI<sub>95%</sub> 1.24–3.52;  $P = 0.02$ ). A strong positive correlation was found between countries being located in Europe and high GNI per capita





**Fig. 3.** Bubble plot for meta-regression of herd-level *Coxiella burnetii* prevalence based on bulk-tank milk with average herd size as continuous covariate. Points represent the seventeen studies that matched the inclusion criteria in the systematic review. Bubble size is in relation to the weight of each primary study.

income ( $r = 0.633$ ,  $P < 0.05$ ), but between location in Europe and compulsory disease notification ( $r = -0.239$ ,  $P = 0.24$ ), and between high GNI per capita and notification ( $r = -0.076$ ,  $P = 0.82$ ) correlations were weak and negative. Herd size was not meaningfully correlated with the origin of the studies ( $r = -0.468$ ,  $P = 0.12$ ), notification ( $r = -0.428$ ,  $P = 0.16$ ), or with GNI per capita ( $r = -0.444$ ,  $P = 0.14$ ).

#### 4. Discussion

Global serological or molecular prevalences from pathogens as diverse as *Toxoplasma gondii* and *Helicobacter pylori* have been estimated by meta-analyses following a systematic review of the published body of studies [56,57]. We conducted a comprehensive keyword-based systematic review of the literature on the global molecular prevalence of *C. burnetii* in bovine BTM samples and data from those studies matching the inclusion criteria was extracted and included in a meta-analysis. For the purpose of this review, only adequately randomised studies with a cross-sectional design were included.

Heterogeneity among studies was first investigated by Higgins'  $I^2$  statistic which indicates the proportion of heterogeneity not due to chance. A high level of heterogeneity ( $\geq 75\%$ ) indicates another source of variability besides the random error. The high  $I^2$  value (98%) led to the choice of a random-effects model for estimating the overall weighted *C. burnetii* herd-level prevalence among eligible articles, which makes no assumption that the prevalence is constant across the studies. The meta-analysis shows that *C. burnetii* is widely distributed in dairy farms around twelve countries from 3 continents (America, Europe, and Asia). The best estimate of global *C. burnetii* herd-level prevalence, based on the studies matching the current inclusion criteria, was 37.0%. While there was no obvious evidence of publication bias based on Egger's test, this test has limited power and the possibility of bias cannot be altogether excluded [58].

Bulk tank milk samples are a widely used approach for studying infectious diseases of dairy livestock at the population level, despite that dry cows and unhealthy animals are not included and hence BTM only provides a partial representation of the herd sanitary status. The analysis of BTM samples represents a suitable and convenient approach for the

investigation of *C. burnetii*, not only for initial farm-level screening in situations where their disease status is unknown, but also for repeated analyses during monitoring programmes or after sanitary interventions such as antibiotic administration [59] or vaccination [60,61]. A positive BTM result confirms herd exposure to *C. burnetii*.

The molecular diagnostic methods of studies included in this meta-analysis targeted different regions of the bacterial *C. burnetii* genome. The repetitive element *IS1111* was selected in most of the published studies as this multiple copy gene is presumed to increase the sensitivity of the test [62]. Other studies used PCRs targeting *com1*, *icd* and 16S rRNA genes. The *com1* element is frequently used for accurate quantification, as this is a single-copy gene [63]. Additionally, the analysis of 16S RNAs may reveal the prevalence of *Coxiella* as a genus, by the identification of both *C. burnetii* and *Coxiella*-like organisms [44].

The overall weighted *C. burnetii* prevalence found in bovine dairy herds was higher than the 5.1% to 22.1% range reported for BTM samples from sheep dairy flocks [47,64,65]. This difference could be explained by the primary route of bacterial transmission in each species. A higher *C. burnetii* prevalence might be expected in bovine milk, which is the predominant route of shedding for cows (and with a longer duration), whereas milk is less important for transmission from goats and sheep [9,23].

Two nationwide studies in Dutch dairy herds revealed markedly different prevalence levels in 2011 (56.6%) and 2014 (18.8%) [25,46], when using the same molecular approach in a similar number of herds. The lower prevalence in 2014 might be related to compulsory control measures applied in dairy goat farms after the large human Q fever outbreak in 2007–2010 [11,66]. There is some albeit limited evidence that the same outbreak strain may affect both cattle and goats in the Netherlands [67], and measures applied to goat farms might have indirectly helped to reduce prevalence in bovine herds. Similarly, three studies conducted in Italian herds in 2013 and 2014 also reported differences in *C. burnetii* prevalence. Valla et al. (2014) [51] revealed a nationwide prevalence of 40.0%, while Vicari et al. (2013) [34] found a higher prevalence of 60.0% in the northwest region of Lombardy, where almost half of Italian cows' milk is produced [68]. The molecular prevalence of *C. burnetii* found in Lombardy represented a marked

increase compared to a previous two-year study (2007–2008) conducted in the same region (40.0%) [50].

Differences in the bacterial shedding patterns among ruminants and uncertainty about the importance of milk-borne infection may result in emphasis on different control measures depending on the species. In small ruminants, the identification of high-risk dams before parturition is important in avoiding zoonotic risk [69]. In cattle where milk is the primary shedding route, pre-partum monitoring may not be as appropriate [69]. Identification of chronic *C. burnetii* milk shedding cattle may be more effective in preventing environmental contamination, decreasing the risks of transmission among animals and preventing the spread of the bacterium.

Only five of the seventeen selected articles included analysis of factors associated with *C. burnetii* infection. Herd size, cattle density and purchasing replacement animals from external sources were all linked with *C. burnetii* infection [43,46]. Additionally, the presence of ticks on cattle was associated with BTM PCR positivity [46].

For both cattle and small ruminants, a positive correlation between herd size and herd prevalence of *C. burnetii* has been reported [70,71]. The association between herd size, density of animals and an enhanced risk of *C. burnetii* infection has been well demonstrated [10,72]. Close contact between cows is an intrinsic characteristic of dairy herd management systems, and larger herds offer even greater chances for contact and transmission. Densely populated farms are prone to a higher risk of transmission of the pathogen within the herd after *C. burnetii* is introduced into the farm. Additionally, high animal density leads to greater bacterial load and thus higher environmental contamination [73], which may represent an increased risk of transmission to either cattle or people. This meta-analysis showed that elevated prevalence of *C. burnetii* is associated with large-sized herds, where the odds of a BTM sample testing positive double with every unit increase in  $\log_e$  herd size (odds ratio  $CI_{95\%} 1.24$ – $3.52$ ). Accordingly, of the moderators analysed, average herd size had the largest effect, accounting 33.0% of the observed level of heterogeneity among studies.

While Q fever has been studied in both European and non-European countries, these two contexts have not previously been contrasted. The overall prevalence of *C. burnetii* infection was remarkably similar in European and non-European studies (both 37%). The greater variability among non-European studies ( $CI_{95\%} 18.0\%$ – $58.5\%$ ) than among European studies ( $CI_{95\%} 22.8\%$ – $52.2\%$ ) could be accounted for by the differences in the numbers of studies and herds investigated.

The mandatory notification of a disease should be helpful not only for early identification of outbreaks but also to enable evaluation of the effectiveness of control strategies. For instance, legislation implemented by the Dutch government in the face of the largest Q fever outbreak ever recorded included compulsory notification of coxiellosis [66]. In the current meta-analysis, a remarkable similarity was noted between overall weighted prevalence of *C. burnetii* in BTM samples from countries with mandatory (37.0%,  $CI_{95\%} 22.3$ – $52.9\%$ ) and non-mandatory (36.9%,  $CI_{95\%} 19.4$ – $56.4\%$ ) notification legislation.

In our meta-analysis, the GNI per capita seems to have a minor effect as a moderator of the prevalence of *C. burnetii* in BTM samples. When the studies were stratified according to this indicator of economic development, high-income countries had twice the overall weighted prevalence of upper-middle income countries, albeit that this difference was not statistically significant ( $P = 0.24$ ). All publications matching the inclusion criteria were conducted in high and upper-middle income countries. None of the studies conducted in low-middle and low-income countries that were identified in the initial search fulfilled the inclusion criteria and were rejected from the meta-analysis. For instance, an ineligible study carried out in Egypt reported a 22% molecular prevalence of *C. burnetii* in individual milk samples [74] and one carried out in Bangladesh reported 15.6% seroprevalence in herd milk specimens [75]. These findings suggest that further field studies could prove rewarding. The overall prevalence in low-middle and low-income countries remains unknown. There is evidence of extensive ruminant infection with

*C. burnetii* throughout African countries where the threat of human exposure and significant economic impact are possibly underestimated [76].

Some heterogeneity might have resulted from methodological variation among nine of the 17 studies that used qPCR to detect the *IS1111* target. Four of these [25,46,49,52] used the TaqVet *Coxiella burnetii* LSI kit and followed the same manufacturer's instructions for the amplification reaction and for the interpretation of the results. These four studies considered samples as positive with a cycle threshold (Ct) < 40. Two further studies used threshold Ct values of 36.5 [53] and 36.95 [54], while the remaining three studies using qPCR to detect *IS1111* did not report threshold Ct values.

Moreover, whereas the *IS1111* transposon-like element is a multi-copy gene [77], the 16S rRNA target used in South Korean study [44] and the *com1* target used in the two studies in Iran [23,45] are both single copy genes. The assays used in these studies might have had lower sensitivity and indeed, the studies using the single copy assays had three of the four lowest prevalence values. All three of these studies were in non-European countries where the disease is notifiable, and the two Iranian studies were in an upper-middle income country, which may have introduced a degree of bias in the analysis.

Although the moderator analysis identified average herd size as one source, most of the heterogeneity remained unexplained (residual heterogeneity  $I^2 = 97.0\%$ ;  $P < 0.01$ ). It is quite possible that other factors, not currently addressed, influence the *C. burnetii* herd-level prevalence. Unsurprisingly, two of the moderators were highly correlated; studies in European and in high-income countries showed a significant and positive correlation ( $r = 0.627$ ,  $P < 0.01$ ). Awareness of the relationships between moderators that may potentially induce bias in the analysis should be considered when drawing conclusions [78].

## 5. Conclusion

This meta-analysis reports a high overall global prevalence of *C. burnetii* in BTM samples of 37.0% ( $CI_{95\%} 25.2$ – $49.5\%$ ), showing widespread herd-level circulation of this agent in bovine dairy farms. These results should be of interest not only for European countries where *C. burnetii* is a well-known health threat, but also in countries where epidemiological investigations have been limited, its importance as a zoonosis may be underestimated and prevention strategies may need to be implemented. Information on local biosecurity practices and environmental conditions would be valuable for a full understanding of *C. burnetii* prevalence globally, but such descriptions were lacking in most of the publications considered in the meta-analysis. While this study has shown the global herd prevalence of *C. burnetii* in dairy cattle to be high, in many countries, including high-income countries such as Belgium, Italy, Portugal and UK, the disease is not currently notifiable, and control is not mandatory. To make it so might represent an additional burden on dairy farmers and would require justification on economic or public health grounds for which further study might be required. The high herd-level circulation of *C. burnetii* in bovine dairy farms in several countries showed by this study reinforces the need for further investigations on this globally important zoonosis.

## Author contributions

AR and MCE conceptualised the study. AR and MF performed the systematic review, including data collection and screening the retrieved records. AR, MCE and LGC conducted the data-analysis. All authors (AR, MF, LGC, KT, FRC and MCE) made contributions to the interpretation of results. All authors participated in the manuscript drafting and reviewing.

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## Declaration of Competing Interest

None declared.

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